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Arsenite oxidizing bacterium isolated from high arsenic groundwater aquifers from Datong Basin, Northern China

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Abstract

A microbial strain (Datong-1) was isolated from high arsenic aquifer sediments from the Datong Basin. The strain Datong-1 was identified as an *Arthrobacter sp*. based on 16S rDNA phylogenetic relationships, and is most closely related to DQ320481.1 and EU 196326.1 within the *Arthrobacter genus*. Batch experiments with sediment samples were carried out in order to study the arsenite oxidizing ability of Datong-1. These experiments indicate that strain Datong-1 can rapidly oxidize As(III) to As(V). Cell growth was concurrent with the variation of concentration levels of As(V) and As(III) in the suspension, indicating the impact of microbial activity on As(III) oxidization. Therefore, the Datong-1 strain, which can oxidize As(III), is a potential candidate for remediating arsenic contaminated groundwater in the Datong Basin in the future.

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Keywords: Groundwater; Arsenite oxidize bacterium; Datong Basin; Arsenic; Speciation.

1. Introduction

Arsenic occurrence in environment can be toxic to human and other organisms and cause severely health problems. The detected high arsenic concentration in groundwater has been caused serious threaten to the health of local residents at Datong Basin. Concentration of arsenic in groundwater from the Datong Basin usually exceeds the WHO recommended value (10 μg/L) for drinking water, with the maximum value up to 1820 μg/L [1]. Because of the inaccessibility of potable surface water resource, groundwater becomes the preferred water source to use for both domestic and agricultural irrigation purposes. Due to the long time consumption of arsenic contaminated groundwater, serious arsenic related endemic has

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prevailed in this area. So in order to mitigate the arsenic threaten and to assure a sustainable groundwater supply, development of efficient arsenic removing technologies is urgently necessary in this area.

Many studies indicate that some bacteria can oxidize As(III) to As(V) in natural environments [2]. Furthermore, As(V) is less mobile than As(III) due to its negative charge and facile adsorption. As(III) is more toxic than As(V), thus the transformation of As(III) to As(V) is an important way to reduce arsenic exposure risk. Due to a effective transformation of As(III) to As(V), arsenite oxidizing bacterium could be potentially used to treat arsenic contaminant aquifers.

The purposes of the current study are to (1) isolate and determine the arsenite oxidizing bacterium from high arsenic aquifer sediments from Datong Basin; (2) and then study the capacity of arsenite oxidizing bacterium to oxidize As(III) to As(V).

2. Study area

The field study area (the Datong Basin) is located in an arid and semi-arid region of Shanxi Province, northern China, with a mean annual rainfall of 300-400 mm and a very high mean evaporation rate of about 2000 mm per year; about 80% of rainfall occurs from July to August. The Datong Basin covers an area of about 6000 km^2 and it is composed of Pliocene to Pleistocene and Holocene unconsolidated sediments with a thickness from 1500 m to 3500 m. Groundwater mainly occurs in two aquifers (shallow and deep groundwater), which mainly consist of Middle to Late Pleistocene and Holocene alluvial-pluvial gravel and sand, with depth less than 150 m below the land surface. High arsenic groundwater mainly occurs in shallow groundwater aquifer with the depth less than 50 m below the land surface.

3. Material and methods

High arsenic aquifer sediments were collected from borehole using rotary drilling method at Datong Basin in August 2008. The sediment samples were immediately sealed in PVC bucket using wax and kept at 4^oC in dark after sampling. Then the samples were transported to laboratory for bacterial, DNA extraction and biochemical study. In order to avoid the contamination of bacteria, the external layer of the samples was removed before carrying out the experiments.

Bacteria were isolated by an enrichment culture method from the arsenic-contaminated sediment samples (As>20 mg/kg). On the basis of their morphological, cultural characteristics, 12 of the 100 isolated arsenic-resistant bacteria were selected for DNA extraction. Phylogenetic analysis was performed for the isolated bacteria using 16S rRNA genes. The phylogenetic tree was constructed by using the neighbour-joining method in the MEGA program version 4.1.

Bacterial arsenite oxidizing ability was examined using chemically defined liquid medium with concentration of 1 mM As(V) and As(III). And then 10 μL 0.01 M $KMnO_4$ was added into the liquid medium to test the oxidizing or reducing abilities of strains. In this experiment, As(III) and As(V) were determined periodically to monitor the oxidation of As(III). The separation of As(III) and As(V) were performed using anion exchange resin (AG 1-X8). The detailed procedure is described by Huntsman-Mapila et al. [3]. And then arsenic concentration was determined by hydride generation atomic fluorescence spectrometry (HG-AFS) (AFS-820, Titan).

4. Results and discussion

4.1. Isolation and identification of Datong-1.

An indigenous strain Datong-1 was isolated and identified from sediment samples (Fig. 1) and the further 16S rDNA study was conducted on it. No fuagellum and loosen structure of cytoderm was observed by microscopy on the isolated Datong-1 strain.

Phylogenetic analysis by 16S rDNA indicates that Datong-1 strain is most closely related to DQ320481.1 and EU 196326.1 with 100 % and 98 % sequence identity, respectively. These two strains are both identified in *Arthrobacter sp.* (Fig. 2). Moreover, Datong-1 strain is negative for oxidase activity, esculin hydrolysis, β-galactosidase activity, glucose fermentation and nitrate reducing and positive for gelatinase activity, and indole formation. These are typical metabolic characteristics of Arthrobacter sp. Therefore, in this study Datong 1 strain is identified as a species in the *Arthrobacter sp*. More recently, two phylogenetically and physiologically distinct species within the Thermus genus, *T. aquaticus* and *T. thermophilus*, have been found to rapidly oxidize As(III) [4]. Moreover, *T. thermophilus* is included in the phylogenetic tree in the current study.

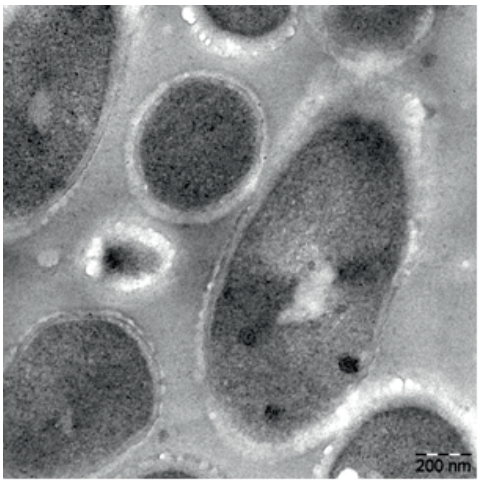

Fig.1. Transmission electron micrograph of Datong-1.

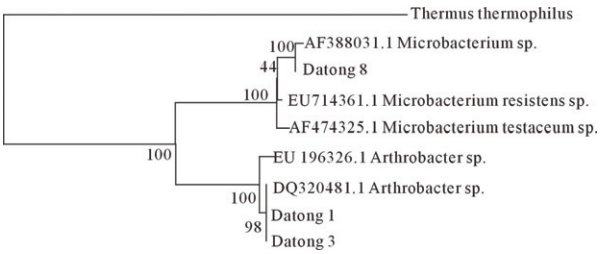

Fig. 2. Phylogenetic tree of Datong-1.

4.2. Arsenite oxidation

Batch experiments examining strain Datong-1 for the ability to oxidize arsenite to arsenate were conducted with shaking for 40 h. Within 12 h after inoculation, we do not observe any significant variation of As(III) and As(V) concentration (Fig. 3A). Due to the low cell density in the first 12 hours (Fig. 3B) biotic oxidation of arsenite cannot be expected. Then the strain Datong-1 grew exponentially for about 10 hours from 15 to 25 h after inoculation and the cell density increased by an average of approximately 10 folds of magnitude (Fig. 3B). Microbial oxidation occurred during exponential growth and resulted in the rapid increase of As(V) concentration and decrease of As(III) concentration (Fig. 3 A and B). After exponential growth, cell density reached stationary state. During the late exponential growth stage, the stable measured a As(III) concentration indicates that oxidation is almost completed. The control experiments without strain inoculation showed no obviously change in arsenic speciation (data not presented).

Cell growth was concurrent with the increase in As(V) concentration and decrease in As(III) concentration. These results provide strong evidence of As(III) oxidation by Datong-1. The transformation of As(III) to As(V) is regarded an arsenic tolerance strategy because As(III) is more toxic than As(V) [5].

Datong-1 as an arsenite oxidizing strain could potentially be applied to clean the arsenic contaminated groundwater at Datong in the future.

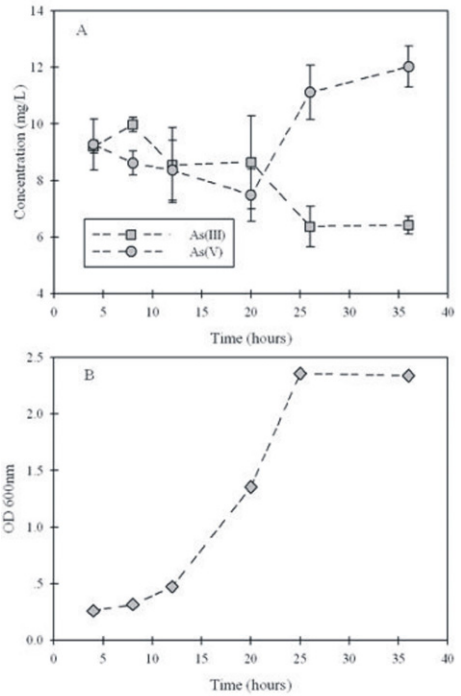

Fig. 3. Oxidation of As(III) to As(V) by Datong-1 (A) and growth curve of strain Datong-1 in CDM without arsenic.

5. Conclusions

Based on 16S rDNA analysis, Datong-1 is identified as a species in the *Arthrobacter sp*. This study suggests that the indigenous bacteria are able to resist to toxic As in the As-contaminated environments and further to oxidize As(III) to As(V), a less toxic form. Our experimental results indicate that Datong strain has several advantages for potential application in bioremediation of As-contaminated aquifers of Datong Basin.

Acknowledgements

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